



KARYOLOGIC AND MOLECULAR ANALYSIS OF *PROECHIMYS* ALLEN, 1899 (RODENTIA, ECHIMYIDAE) FROM THE AMAZONIAN REGION ¹

(With 3 figures)

CIBELE R. BONVICINO ^{2, 3, 4}

IVONE B. OTAZÚ ^{3, 5}

JÚLIO F. VILELA ^{3, 6}

ABSTRACT: Karyologic and molecular analyses were carried out in *Proechimys quadruplicatus* and two other *Proechimys* species from the northern bank of the Rio Negro, Brazil. Analyses of cytb DNA sequence data and karyologic attributes partially sustained the *goeldii* species group. Molecular analyses grouped the two *Proechimys* sp. A haplotypes here sequenced with other specimens from the Amazonian region of Brazil and Venezuela, suggesting that they belonged to a single taxon. The three specimens of *Proechimys* sp. B also formed a monophyletic group. *Proechimys* sp. A, *Proechimys* sp. B, and *P. guyannensis* were grouped by karyologic and/or molecular data indicating that they are very similar one another and belong to the same species group, the *guyannensis* group. Phylogeographic analyses showed a high geographic structuration in the *Proechimys* sp. A population and the presence of a median vector between haplotypes of different rivers suggested that the large Amazonian rivers are barrier to these population.

Key words: Karyotype, phylogeny, *Proechimys*, Amazon, cytochrome *b*.

RESUMO: Análise cariológica e molecular de *Proechimys* Allen, 1899 (Rodentia, Echimyidae) da região Amazônica.

Análises cariológicas e moleculares foram realizadas em *Proechimys quadruplicatus* e duas outras espécies de *Proechimys* da margem norte do rio Negro, Brasil. Análises da seqüência de ADN do citocromo *b* e dos atributos cariológicos sustentam parcialmente o grupo de espécies *goeldii*. As análises moleculares agruparam os dois haplótipos de *Proechimys* sp. A aqui seqüenciados com outros espécimes da região Amazônica do Brasil e Venezuela sugerindo que eles pertençam ao mesmo táxon. Os três espécimes de *Proechimys* sp. B formam um grupo monofilético. *Proechimys* sp. A, *Proechimys* sp. B e *P. guyannensis* se agrupam pelos dados moleculares e/ou cariológicos indicando que eles são bastantes similares e pertencem ao mesmo grupo de espécies, o grupo *guyannensis*. A análise filogeográfica mostrou um padrão de estruturação geográfica forte nas populações de *Proechimys* sp. A, e a presença de vetores médios entre os haplótipos de diferentes rios, na análise de "median - joining", sugere que estes rios sejam barreiras para estas populações.

Palavras-chave: Cariótipo, filogenia, *Proechimys*, Amazonas, citocromo *b*.

INTRODUCTION

The genus *Proechimys* Allen, 1899 shows a drastic variation in diploid chromosome number, ranging from $2n=14$ to 62 (BARROS, 1978; REIG & USECHE, 1976), and some authors have directly tied this diversity to speciation in the genus (REIG & USECHE,

1976). Moreover, karyologic data have also been valuable for a precise identification of *Proechimys* species (PATTON & GARDNER, 1972), which was the first paper to show that karyologic differences could be used to diagnose species and that each karyotypic form was morphologically unique. Conversely, morphologic analyses lead to controversial taxonomic

¹ Submitted on May 20, 2004. Accepted on January 27, 2005.

² Fundação Instituto Oswaldo Cruz, Departamento de Medicina Tropical, Laboratório de Biologia e Controle da Esquistossomose. Av. Brasil, 4365, Manguinhos, 21-45-900, Rio de Janeiro, RJ, Brasil.

³ Instituto Nacional de Câncer, Divisão de Genética. Rua André Cavalcanti, 37, 4º andar, 20231-050, Rio de Janeiro, RJ, Brasil.

⁴ Fellow of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

⁵ Hospital Universitário Clementino Fraga Filho/UFRJ. Ilha do Fundão, Rio de Janeiro, RJ, Brasil.

⁶ Museu Nacional/UFRJ, Departamento de Vertebrados. Quinta da Boa Vista, São Cristóvão, 20940-040, Rio de Janeiro, RJ, Brasil.

arrangements for the genus *Proechimys* as well as disparate criteria for defining subordinate taxa consequently to difficulties in identifying discrete morphological features. As postulated by THOMAS (1928), these rodents are characterised by an extensive morphological variability with overlapping morphological characters between different taxa. PATTON (1987), based on a morphological criterion, considered that *Proechimys sensu stricto* comprised nine species groups. Recently, another four *Proechimys* species, *P. kulinae*, *P. gardneri*, *P. pattoni*, and *P. echinothrix* have been described by DA SILVA (1998) and one species, *P. oris* Thomas, 1904, was considered junior synonym of *P. roberti* Thomas, 1901 (WEKSLER *et al.*, 2001).

In this paper we describe a new karyologic variant of *Proechimys quadruplicatus* Hershkovitz, 1948 and two new karyotypes of other two *Proechimys* species and comment on the role of karyology in species identification. We also analysed the phylogenetic relationships of these karyologic forms in respect to other *Proechimys* species using the mitochondrial gene cytochrome *b* (*cytb*) sequence data.

MATERIAL AND METHODS

Proechimys specimens were collected in eight Brazilian localities, in tributaries of the left bank of Rio Negro in the states of Amazonas (localities 1, 2, 3, 4 in Barcelos and localities 5, 6, 7 in Santa Isabel) and Roraima (locality 8, Fig.1). Additionally, sequences of species from locality 9 and 10, kindly provided by Dr. James L. Patton (JLP, University of California, Berkeley, USA), were used in molecular analyses. "Igarapé" refers to a small stream. Skins and skulls of CRB specimens are housed in the mammal collection of Museu Nacional - Rio de Janeiro (MN). The following acronyms refer to field numbers: (CRB) C.R.Bonvicino M.N.F. da Silva (MNFS), J.L.Patton (JLP), and A.L.Gardner (ALG). Acronyms U, AJ, and AY refer to accession numbers of GenBank.

Proechimys sp. A – State of Amazonas, Barcelos Municipality, (1) right bank of Rio Curuduri, Igarapé Tucunaré 00°09'89"N 63°30'49"W (♀ MN69019, 69020, 69022 69024, 53292, 69025, 69026, 69028, 63289; ♂ MN69017, all karyotyped); (2) right bank of Rio Curuduri, Igarapé

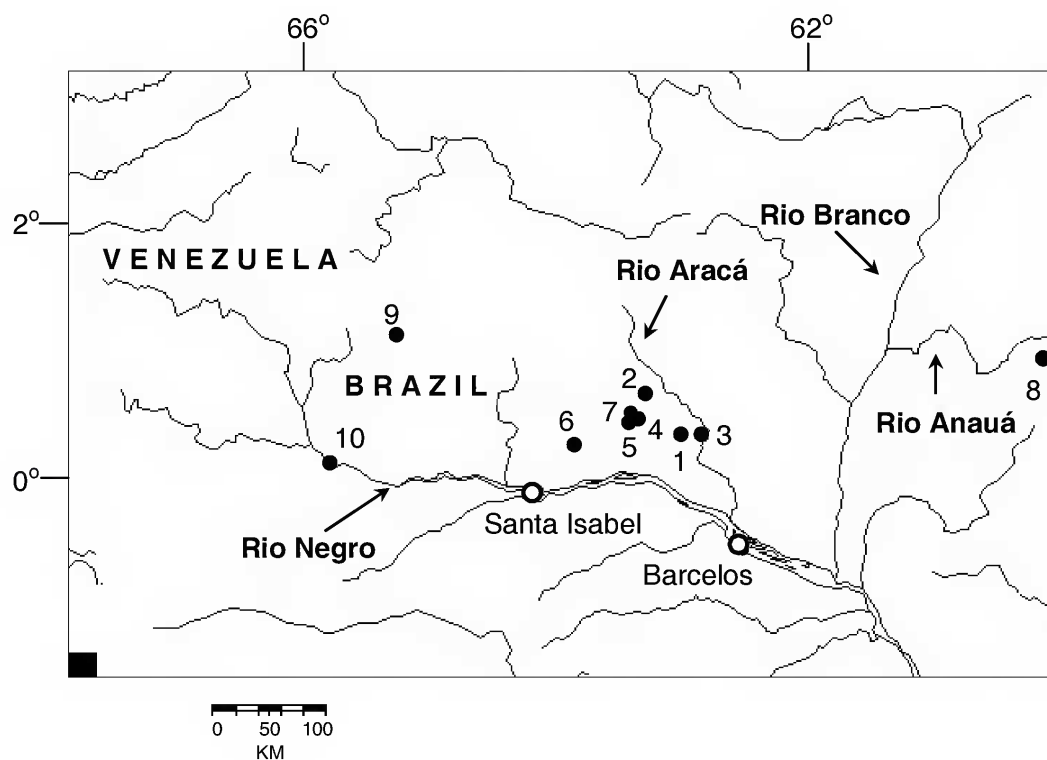


Fig.1- Localities of gather of *P. quadruplicatus* and *Proechimys* sp. A specimens analysed in this study. Brazil: State of Amazonas (1) Igarapé Tucunaré and (2) Igarapé Curudurizinho, tributaries of Rio Curuduri; (3) Igarapé do Bigorna, tributary of Rio Aracá; (5) Igarapé Ucuqui, (7) Igarapé Acuquaia and (4) Igarapé Japomeri, tributaries of Rio Paduari; (6) Igarapé Araújo, tributary of Rio Preto, (10) Comunidade Colina, São Gabriel da Cachoeira, right bank of Rio Tiquié; State of Rondônia (8) São João da Baliza. Venezuela: (9) Rio Mawarinuma, Amazonas.

Curudurizinho 00°34'09"N 63°52'01"W (♀ MN69050-69052, ♂ MN69053, all karyotyped); (3) right bank of Rio Aracá, right bank of Igarapé do Limão, Igarapé do Bigorna 00°09'52"N 63°15'43"W (♀ MN69033, 69034, 69038, 69040-69042, 69392, 69057, ♂ MN69032, 69035-69037, 69039, 69044, 69059 and CRB 1796, fifteen of which karyotyped, Cyt *b* sequence data from CRB1796, obtained by Dr. J.L.Patton, was used in molecular analyses); (4) left bank of Rio Padauari, Igarapé Japomeri 0°20'51"N 64°00'28"W (♀ MN69138), Santa Isabel Municipality, (5) left bank of Rio Padauari, Igarapé Ucuqui 00°18'50"N 64°01'40"W (♀ MN69001, 69004, 69008-69011, 69014, ♂ MN69005, 69012, 69015, 53291, 69017, all karyotyped), (6) left bank of Rio Preto, Igarapé Araújo 00°04'04"N 64°35'41"W (sex indetermined: MN69127, ♀ MN69124, 69128, 69131), (7) Rio Padauari, Igarapé Acuquaia 00°20'28"N 63°57'12"W (♀ MN69049, karyotyped).

P. quadruplicatus – State of Amazonas, Santa Isabel Municipality, (5) left bank of Rio Padauari, Igarapé Ucuqui 00°18'50"N 64°01'40"W (♀ MN69013, ♂ MN69003; CRB1483, all karyotyped; *cytb* from CRB1483 and MN69007 was sequenced by us and used in molecular analyses).

Proechimys sp. B – State of Roraima, São João da Baliza Municipality, (8) UHE Alto Jatapú 00°57'01"N 59°54'40"W (♀ MN68174, 61642, ♂ MN61643, all karyotyped, *cytb* sequences of this species were obtained by Dr. J.L.Patton, ♂ CRB635).

Chromosome preparations were obtained from bone marrow cultures in RPMI 1640, 20% foetal calf serum, colchicine (10^{-6} M) and ethidium bromide (5µg/ml) for two hours. This latter reagent is used to elongate the chromosomes (IKEUCHI, 1984). C- and G-banding were carried out as described by SUMNER (1972) and SEABRIGHT (1971) respectively.

DNA of specimens CRB1483 and MN69007 belonging to two *Proechimys* species was isolated from liver tissue fragments preserved in ethanol (Tab.1) following the procedures of SAMBROOK, FRITSCH & MANIATIS (1989). Cytochrome *b* DNA (ca. 472 bp) was amplified with primers MVZ 05 and MVZ 16 (SMITH & PATTON, 1993) and sequenced with an ABI Prism™ 377 automatic DNA sequencer. Sequences were manually aligned. NETWORK software (BANDELT, FOSTER & RÖHL, 1999; POSADA & CRANDALL, 2001) was used to analyze intraspecific phylogenies, and to evaluate the population structure and geographic distribution pattern of *Proechimys* sp. A. This analysis was carried out only with *cytb* variable

sites. Maximum parsimony trees were obtained through heuristic searches using the tree-bisection-reconnection branch-swapping algorithm in PAUP* 4.0b10 (SWOFFORD, 1999), with all sites equally weighted. Bootstrap values were calculated by heuristic search based on 1,000 replicates.

In addition to *cytb* sequence data from specimens collected by us, sequence data were obtained in GenBank, WEKSLER *et al.* (2001) and PATTON, DA SILVA & MALCON (2000). The following specimens were used in molecular analyses: *P. quadruplicatus* (U35413, São Carlos do Rio Negro, AM, Venezuela, listed as *P. amphichoricus* (Moojen, 1948) by PATTON, DA SILVA & MALCON, 2000), *P. cuvieri* Petter, 1978 (U251402 from French Guiana), *P. guyannensis* E. Geoffroy, 1803 (AJ251395, AJ251396, AJ251397, AJ251398, AJ251399, AY206600, AY206601, AY206602 from French Guiana, listed as *P. cayennensis* (Desmarest, 1817, ICZN, 2002), *P. roberti* (LHE512, Rio Xingú, Brazil), *P. brevicauda* (Günther, 1877)(JLP 8271, Amazonas, Perú); *P. simonsi* Thomas, 1900 (JLP 15874 from Barro Vermelho 06°28'S 68°46'W, left bank of Rio Juruá, AM, Brazil); *P. steerei* Goldman, 1911 (JLP 15705 from Seringal Condor 06°45'S 70°51'W, left bank of Rio Juruá, AM, Brazil); *P. cuvieri* (U251402), *Proechimys* sp. A (INPA 2433, 2534 from Comunidade Colina, right bank of Rio Tiquié, São Gabriel da Cachoeira ca. 0°07'49"S 67°05'21"W, AM, Brazil; ALG 14242, 14255, 14282, 14297 from ca. 2km SE and in Neblina base camp, Rio Mawarinuma ca. 01°11'N 66°25'W, Amazonas, Venezuela). *Mesomys hispidus* (Desmarest, 1817) (MNFS 436 from Rio Juruá, Amazonas, Brazil), *Trinomys graciosus bonafidei* (Moojen, 1948)(AF194330) and *Trinomys graciosus graciosus* (Moojen, 1948) (AF194329) were used as outgroups in molecular analyses.

RESULTS

KARYOTYPIC VARIATION

Karyotypic analysis of three specimens of *P. quadruplicatus* showed $2n=28$, $FN=42$. The autosome complement is composed of eight pairs of biarmed chromosomes (two large, five medium-sized and one small pair) and five acrocentric pairs (one medium sized and four small pairs). The X chromosome is a medium sized acrocentric and the Y chromosome is a small acrocentric (Fig.2A). A G-band karyotype is shown in figure 2D. C banding (not shown) showed pericentromeric heterochromatin in the sex

Table 1. Described karyotypes of the species of *Proechimys* here analysed.

TAXA	2n	FN	LOCALITY	SOURCE
<i>GUYANNENSIS</i> GROUP				
<i>Proechimys</i> sp.A	38	52	BR: AM, Barcelos and Santa Isabel	This study
<i>Proechimys</i> sp.B	46	50	BR: RR, São João da Baliza	This study
<i>Proechimys</i> sp.1	28	50-51	Peru: 44km de Pucalppa	ANISKIN (1994)
<i>Proechimys</i> sp.2	30	50	Peru: 39km of Iquitos, Aupauayo	ANISKIN (1994)
<i>Proechimys</i> sp.	44	52	BR: AM, Manaus	LEAL-MESQUITA (1991)
<i>P. guyannensis</i>	40	56	Venezuela	PATTON & GARDNER (1972)
<i>P. guyannensis</i>	40	54	French Guiana: Cayenne and Saul	REIG, TRAINER & BARROS (1979)
<i>CUVERI</i> GROUP				
<i>P. cuvieri</i>	28	46	BR: AC and AM	MAIA & LANGGUTH (1993), PATTON, DA SILVA & MALCON (2000)
<i>P. cuvieri</i>	28	50	French Guyana: Cayenne; BR: AM	REIG, TRAINER & BARROS (1979), PATTON, DA SILVA & MALCON (2000)
<i>GOELDII</i> GROUP				
<i>P. steerei</i>	24	40-42	Peru: Dept Ucayali, Madre de Dios and Loreto; BR: AC and AM	REIG & USECHE (1976), PATTON & GARDNER (1972), GARDNER & EMMONS (1984), PATTON, DA SILVA & MALCON (2000)
<i>P. goeldii</i>	24	43-44	BR: PA; Peru, Dept Ucayali	PATTON, DA SILVA & MALCON (2000), ANISKIN (1994)
<i>P. quadruplicatus</i> (listed as <i>P.amphichoricus</i> -topotypes)	26	44	VEN: Territorio Federal Amazonas	REIG & USECHE (1976)
<i>P. quadruplicatus</i>	28	44	Ecuador: Limoncocha; Peru: Santiago	GARDNER & EMMONS (1984)
<i>P. quadruplicatus</i>	28	42	Peru, Amazonas; BR: AM	PATTON, DA SILVA & MALCON (2000), This study
<i>LONGICAUDATUS</i> GROUP				
<i>P. brevicauda</i>	28	48	Peru; BR: AC	GARDNER & EMMONS (1984), PATTON, DA SILVA & MALCON (2000)
<i>P. aff. brevicauda</i>	28	50-51	Peru: Dept Ucayali	ANISKIN (1994)
<i>SIMONSI</i> GROUP				
<i>P. simonsi</i>	32	58	Ecuador; S Peru; BR: AM	GARDNER & EMMONS (1984), PATTON, DA SILVA & MALCON (2000)
<i>P. aff. simonsi</i>	32	57-58	Peru: Ucayali and Loreto	ANISKIN (1994)

Species were grouped according to PATTON (1987). (VEN) Venezuela, (BR) Brazil, states: (AC) Acre, (AM) Amazonas, (PA) Pará, (RR) Roraima.

chromosomes, in five pairs of biarmed autosomes (numbers 1, 2, 3, 5, 6) and in an interstitial region in the mid part of the largest acrocentric pair (number 7). One medium sized metacentric pair (number 8) lacked pericentromeric heterochromatin, showing a heterochromatic region at the long arm telomere. In one specimen, a heterochromatic region in the long arm telomere of chromosome no. 1 was also observed.

Karyotypic analysis of 16 specimens of *Proechimys* sp. A showed 2n=38, FN=52 (Fig.2B). The autosome complement is composed by 8 pairs of biarmed chromosomes (three large metacentric, one medium-large sized metacentric and four small pairs) and ten pairs of acrocentric chromosomes. The X chromosome is a medium sized acrocentric and the Y chromosome is a small acrocentric. With conventional Giemsa staining both

members of pair number 5 showed a constriction at the distal region of the long arm. A G band karyotype of *Proechimys* sp. A is shown in figure 2E. Karyologic analysis of three specimens of *Proechimys* sp. B showed $2n=46$, $FN=50$ (Fig.2C). The autosome complement is composed by three small sized biarmed pairs and 19 acrocentric pairs varying gradually from large to small. The X chromosome is a medium sized acrocentric chromosome and the Y

chromosome a small acrocentric. With conventional Giemsa staining both members of the largest submetacentric pair showed a constriction at the distal region of the long arm. C banding (not shown) revealed pericentromeric heterocromatin in the sex chromosomes, in three pairs of biarmed autosomes and all acrocentrics pairs, and in an interstitial region at the sub terminal region of the largest acrocentric pair.

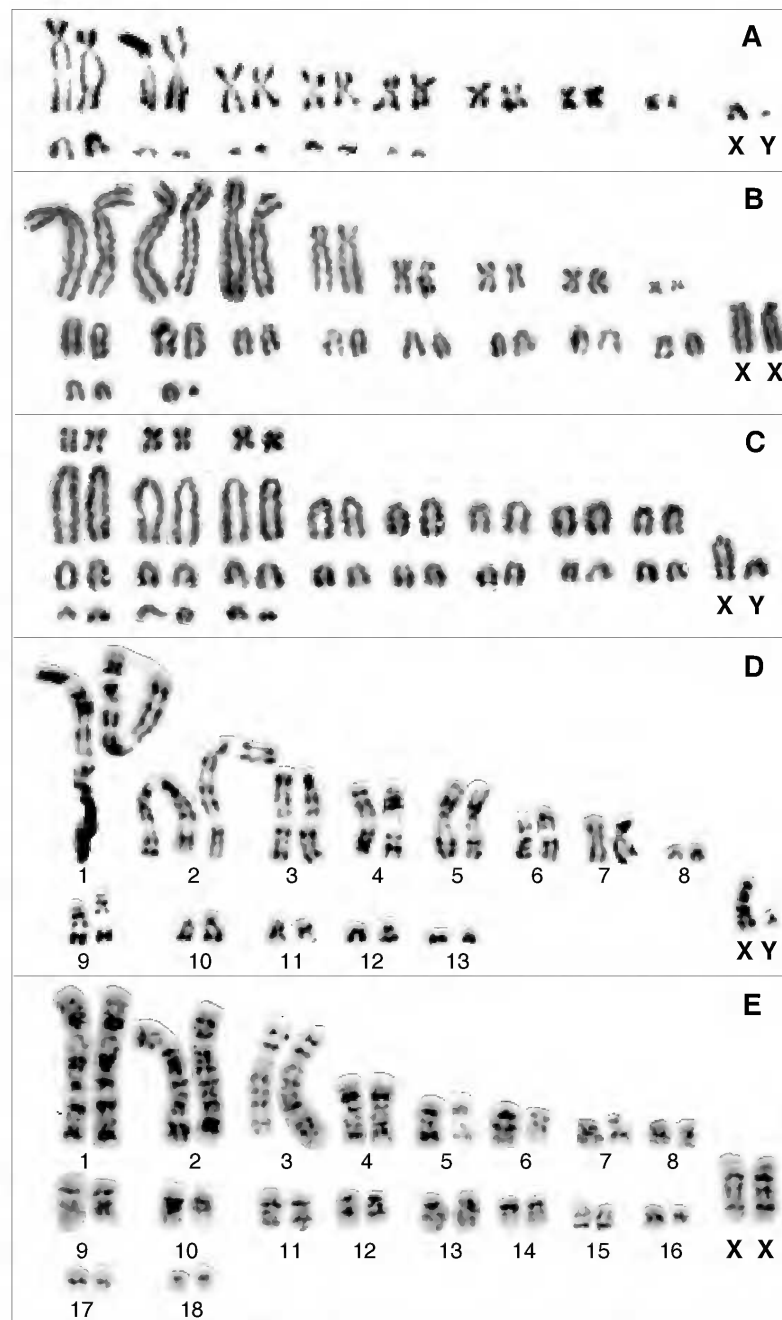


Fig.2- Conventional staining of (A) *P. quadruplicatus* with $2n=28$, $FN=42$ (♂ MN69003), (B) *Proechimys* sp. A with $2n=38$, $FN=52$ (♀ MN69042), (C) *Proechimys* sp. B with $2n=46$, $FN=50$ (♂ CRB635), G - band karyotype of (D) *P. quadruplicatus* (♂ CRB 1483), and (E) *Proechimys* sp (♀ MN69020). X= X chromosome, Y= Y chromosome.

MOLECULAR DATA

Cytochrome *b* data of specimens here sequenced were deposited in GenBank (GenBank 308435 and 308436). Inter-individual variation was observed in *Proechimys* sp. A, *Proechimys* sp. B, and *P. guyannensis*. Divergence levels between *Proechimys* sp. A (2n=38) and *Proechimys* sp. B (2n=46) are considerably greater (p-distance>0.04 in comparisons of any pair of haplotypes) than within either clade (0.03 or less). Divergence levels between *Proechimys* sp. A (2n=38) and *P. guyannensis* are slightly greater (p-distance>0.03 in comparisons of any pair of haplotypes) than within either clade (0.03 or less). Likewise, divergence levels between *Proechimys* sp. B (2n=46) and *P. guyannensis* are also slightly greater (p-distance>0.02 in comparisons of any pair of haplotypes) than within either clade (0.01 or less). Differences between inter-specific haplotypes were generally higher than 10%, except when comparing members of the same specie group (*sensu* PATTON, 1987). In such cases distances are normally greater than 4,0%.

Maximum parsimony (MP) analyses grouped all *Proechimys* species in one monophyletic clade and place *P. roberti* as the most basal offshoot (Fig.3A). *P. quadruplicatus*, *Proechimys* sp. A and *Proechimys* sp. B fall into three clearly defined molecular clades, each of which corresponds to one of the three karyotypes. The clades formed by *Proechimys* sp. A, *Proechimys* sp. B, and *P. guyannensis* haplotypes form a well supported group (94% bootstrap). The latter two are more closely related, however with a lower bootstrap support (68%). The Rio Tiquié *Proechimys* sp. A specimens are placed in a monophyletic clade with the others, with specimens of the same species supported by a bootstrap value of 99%.

Median-joining network analysis showed a more structured geographic pattern than maximum parsimony analyses for *Proechimys* sp. A since a median vector was postulated between haplotypes of each river (Fig. 3B).

DISCUSSION

KARYOLOGIC COMPARISONS

The *Proechimys quadruplicatus* karyologic variant herein described is similar to all species of the *goeldii* species group (*sensu* PATTON, 1987, see Table 1). They share the same diploid and fundamental numbers with other specimens of *P. quadruplicatus* from Brazilian and Peruvian Amazon (PATTON, DA SILVA & MALCON, 2000). However, the karyotype

herein analysed, despite sharing the same diploid number (2n=28) with *P. quadruplicatus* specimens from Ecuador and Santiago in Peru (Tab.1), differed from them in fundamental number and chromosome morphology. The *P. quadruplicatus* karyotype here described showed a medium-sized acrocentric pair that, in Peruvian specimens from Santiago, corresponded to a medium-sized metacentric pair. These results showed the need of further investigations to verify whether these karyologic variations are fixed or polymorphic. With G-band pattern, chromosome pairs numbers 1, 2, 3 and 4 of *P. quadruplicatus* here analysed were recognised as the respective homologues to chromosome pairs numbers 11, 1, 2 and 5 of the karyomorphotype 2n=24, FN=43-44. This karyological variant was tentatively identified as belonging to "*P. steerei*?" by ANISKIN (1994) and was referred to *P. goeldii* Thomas, 1905 by PATTON, DA SILVA & MALCON (2000). The chromosome complement of the *goeldii* species group (*sensu* PATTON, 1987) is characterised by diploid numbers varying from 24 to 28 with fundamental (autosome) numbers ranging from 40 to 44. This species group occurs in the Amazonian region of Peru, Ecuador, Venezuela, and Brazil.

The *Proechimys* sp. A karyotype (2n=38, FN=52) was also different from all other karyotypes previously described in *Proechimys* species. Karyologic data did not allow us to allocate this species to any of the described species groups defined in PATTON (1987) but morphologic traits suggested that this species belong to the *guyannensis* species group (*sensu* PATTON, 1987). Karyotypic comparisons with two species karyotyped by ANISKIN (1994) showed interspecific homologies between chromosome pairs 1, 2 and 3 of *Proechimys* sp. A and chromosome pairs 1, 10 and 11 of *Proechimys* sp.1 (2n=28, see table 1) and *Proechimys* sp.2 (2n=30, see Tab.1 and ANISKIN, 1994). These karyotypes also shared the same fundamental autosome number (FN=50). The 2n=46, FN=50 karyotype herein described in *Proechimys* sp. B specimens was very different from any other previously reported for *Proechimys* species due to its low ratio of biarmed: acrocentric pairs, resulting in unusual low number of autosome arms when compared to other *Proechimys* species (see Tab.1). Despite phylogenetically close related, based on the molecular analyses, the karyologic complement of *Proechimys* sp. B (2n=46, FN=50) differ from *P. guyannensis* (2n=40, FN=54) from French Guyana. The former showed only three biarmed pairs, whereas the latter showed eight biarmed pairs. To derive the *P. guyannensis* karyotype (from French

Guyana) from *Proechimys* sp. B karyotype we need three centric fusions and two inversion events. These karyologic data greatly suggested that these taxa belong to two evolutive lineages.

Proechimys quadruplicatus here analysed showed telomeric, pericentromeric, and interstitial heterochromatin. In the genus *Proechimys*, presence of pericentromeric heterochromatin in most autosomes was found to be widespread (ANISKIN, 1994; MAIA & LANGGUTH, 1993) contrary to scarce telomeric heterochromatin (BUENO & GOMEZ-LAVERDE, 1993; GOMEZ-LAVERDE, BUENO & CADENA, 1990). C-band variants in *P. quadruplicatus* were found to occur in the homozygote condition (both chromosomes with or without heterochromatin) probably due to a polymorphism, despite an apparent lack of heterozygotes.

Proechimys species are generally sympatric, but taxa belonging to the same species group (and sharing similar karyotypes) were not sympatric as we would expect in an allopatric model of chromosome speciation. Extreme variations in heterochromatin, diploid and fundamental numbers in *Proechimys* species pointed to the relevance of karyotypic rearrangements in speciation and to the usefulness of cytotaxonomic studies. Furthermore, groups formed by species sharing karyologic similarities are coincident with the one sharing morphologic similarities.

MOLECULAR ANALYSES

Estimates of inter-specific sequence divergence were generally high (Tab.2), however these values are particular for each *Proechimys* taxa.

The monophyly of the *goeldii* group (*sensu* PATTON, 1987) was supported by karyologic data but not by parsimony analyses. This result confirmed previous analyses based on a larger number of taxa and longer sequence fragments which did not support the monophyly of Patton's *goeldii* group (DA SILVA, 1998).

Proechimys sp. A specimens from different localities (Mawarinuma, Tiquié, Padauari, and Aracá rivers) formed a well supported clade in MP analyses (93% bootstrap values), indicating the monophyly of this group. *Proechimys* sp. B haplotypes also formed a monophyletic group, as expected to occur with specimens belonging to the same taxon. Specimens of *Proechimys* sp. A, *P. guyannensis* and *Proechimys* sp. B grouped in a well - supported clade (bootstrap value of 94%), indicating close phylogenetic

affinities between these taxa. These results confirm the morphologic traits that suggested that *Proechimys* sp. A belong to *guyannensis* group (*sensu* PATTON, 1987). However, the *P. roberti* haplotype here analysed did not cluster with the remaining species of the *guyannensis* group.

The position of specimens from Rio Tiquié with respect to the other *Proechimys* sp. A specimens in the median joining analyses is coherent with geographic data, because the Rio Tiquié specimens are separated from the others by a wide water course, the Rio Negro (Fig.1). This analysis showed a high geographic structuration in the *Proechimys* sp. A population and the presence of a median vector between haplotypes of different rivers suggested that the large Amazonian rivers represent barriers to gene flow between these populations. However, isolation by distance cannot be ruled out in view that riverine effects affected only *P. echinothrix* but not other species like *P. stereei* and *P. simonsi* (MATOCQ, PATTON & DA SILVA, 2000). MP analyses grouped *Proechimys* sp. A specimens from different localities; two of these specimens have the same G-banded chromosome complement and belonged to the same species, suggesting that this new and undescribed species has a wide distribution, with well geographically structured populations.

MORPHOLOGIC COMPARISONS

Comparisons of *Proechimys* sp. B with the illustration of incisive foramen (PATTON, 1987) and part of the ventral part of skull of topotypes of *Proechimys guyannensis arabupu* Moojen, 1948 (PATTON, 1987) showed that these taxa share similar morphology, suggesting that they belong to the same species. On the other hand, molecular and karyological data showed that *P. guyannensis* and *Proechimys* sp. B (= *P. g. arabupu*) are independent evolutive lineages. These morphological similarities suggested that the name *Proechimys arabupu*, whose type locality is Boa Vista, State of Roraima, is available to *Proechimys* sp. B populations.

Karyologic analysis grouped *Proechimys* sp. A with two other *Proechimys* species of Peru and molecular analyses grouped *Proechimys* sp. A, *Proechimys arabupu* (= *Proechimys* sp. B) and *P. guyannensis* suggesting that these species are very related, probably belonging to a single species group, the *guyannensis* group, as defined by PATTON (1987).

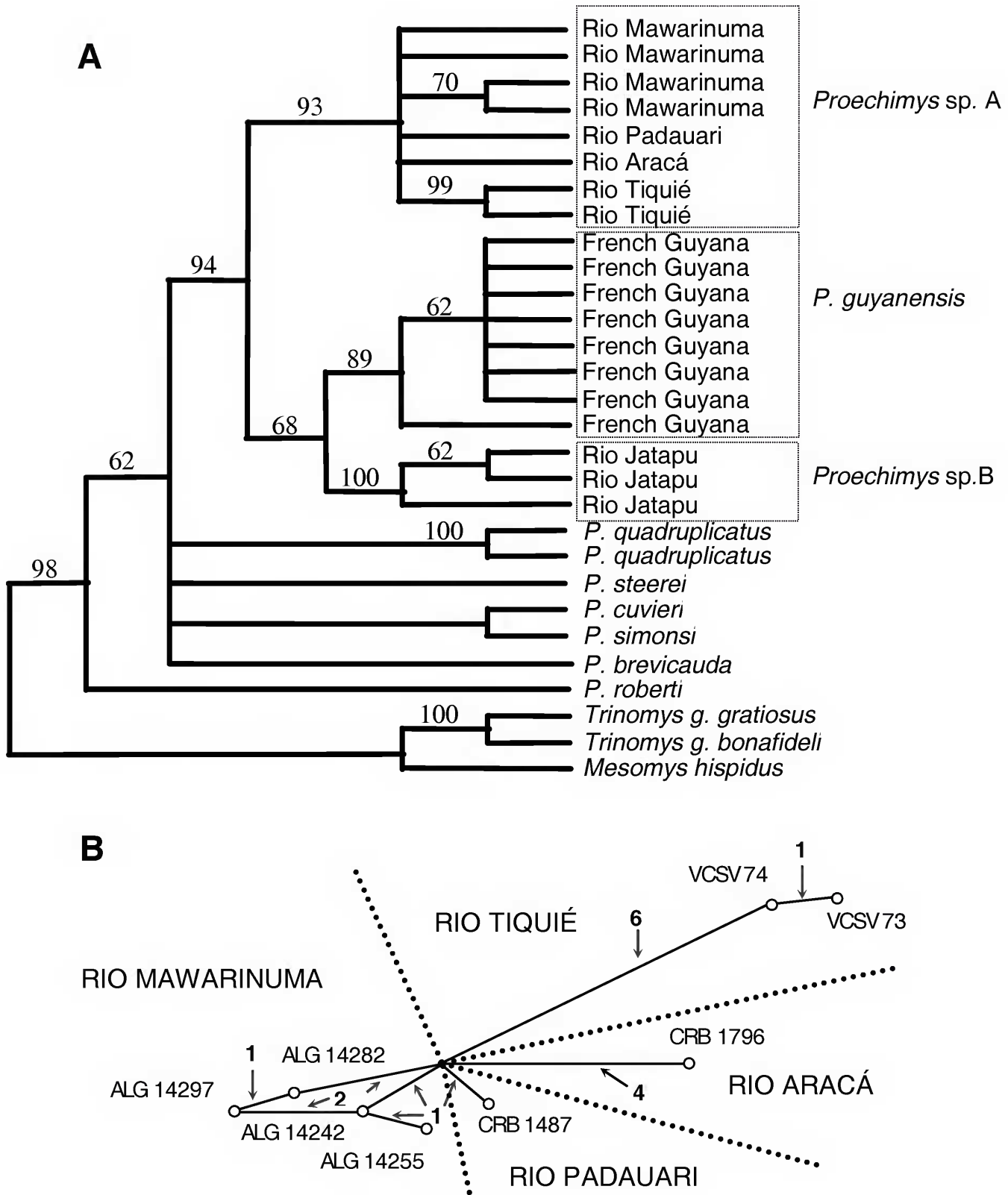


Fig.3- Molecular analyses showing phylogenetic relationships between *Proechimys* specimens: (A) Consensus parsimony tree (length=325, Consistency Index=0.529); (B) median-joining analysis. (○) haplotypes, (●) median vector, (→) number of nucleotide substitution.

Table 2. Distance p estimates between haplotypes. Numbers in bold are distance between specimens of the same species and locality, and shaded case are distance between specimens of the same taxon.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
1. <i>Proechimys</i> sp.A																										Venezuela, Rio Mawarinuma - ALG14242	
2. <i>Proechimys</i> sp.A	.00																									Venezuela, Rio Mawarinuma - ALG14255	
3. <i>Proechimys</i> sp.A	.01	.01																								Venezuela, Rio Mawarinuma - ALG14282	
4. <i>Proechimys</i> sp.A	.01	.01	.00																							Venezuela, Rio Mawarinuma - ALG14297	
5. <i>Proechimys</i> sp.A	.01	.02	.02	.02																						Brazil, rio Aracá, Ig. Bigorna - CRB1796	
6. <i>Proechimys</i> sp.A	.01	.01	.01	.01	.01																					Brazil, Rio Padauari - CRB1487	
7. <i>Proechimys</i> sp.A	.02	.02	.02	.03	.03	.02																				Brazil, Rio Tiquié - VCSV73	
8. <i>Proechimys</i> sp.A	.02	.02	.02	.02	.03	.02	.00																			Brazil, Rio Tiquié - VCSV74	
9. <i>P. guyannensis</i>	.04	.04	.05	.05	.05	.05	.06	.06																		French Guyana - AJ251399	
10. <i>P. guyannensis</i>	.04	.04	.05	.05	.05	.05	.06	.06	.00																	French Guyana - AJ251395	
11. <i>P. guyannensis</i>	.04	.04	.05	.05	.05	.05	.06	.06	.00	.00																French Guyana - AJ251396	
12. <i>P. guyannensis</i>	.04	.04	.05	.05	.05	.05	.06	.06	.00	.00	.00															French Guyana - AJ251397	
13. <i>P. guyannensis</i>	.05	.05	.06	.06	.05	.06	.07	.06	.01	.01	.01	.01														French Guyana - AJ251398	
14. <i>P. guyannensis</i>	.04	.04	.05	.05	.05	.05	.06	.06	.00	.00	.00	.00	.01													French Guyana - AY206600	
15. <i>P. guyannensis</i>	.04	.04	.05	.05	.05	.05	.06	.06	.00	.00	.00	.00	.01	.00												French Guyana - AY206601	
16. <i>P. guyannensis</i>	.04	.04	.05	.05	.04	.05	.06	.05	.00	.00	.00	.00	.01	.00	.00											French Guyana - AY206602	
17. <i>Proechimys</i> sp.B	.06	.06	.07	.07	.07	.07	.05	.05	.04	.04	.04	.04	.05	.04	.04	.03										Brazil, Rio Jatapu - MN68174	
18. <i>Proechimys</i> sp.B	.06	.06	.06	.06	.07	.06	.05	.05	.03	.03	.03	.03	.04	.03	.03	.03	.01									Brazil, Rio Jatapu - MN61642	
19. <i>Proechimys</i> sp.B	.06	.06	.07	.06	.07	.06	.05	.05	.03	.03	.03	.03	.04	.03	.03	.03	.01	.01								Brazil, Rio Jatapu - MN61643	
20. <i>P. quadruplicatus</i>	.12	.12	.13	.13	.12	.12	.13	.12	.12	.12	.12	.12	.12	.12	.12	.11	.12	.12	.12								
21. <i>P. quadruplicatus</i>	.13	.13	.14	.14	.13	.13	.14	.13	.13	.13	.13	.13	.13	.13	.13	.12	.13	.12	.12	.02							
22. <i>P. steerei</i>	.11	.11	.12	.11	.11	.11	.13	.12	.09	.09	.09	.09	.10	.09	.09	.09	.10	.10	.10	.10							
23. <i>P. cuvieri</i>	.11	.11	.12	.11	.12	.12	.13	.13	.11	.11	.11	.11	.12	.11	.11	.11	.12	.12	.12	.13	.14						
24. <i>P. brevicauda</i>	.13	.13	.14	.13	.13	.13	.14	.14	.13	.13	.13	.13	.14	.13	.13	.13	.13	.13	.13	.12	.14	.12					
25. <i>P. simonsi</i>	.11	.11	.11	.11	.12	.12	.13	.13	.12	.12	.12	.12	.13	.12	.12	.12	.13	.13	.13	.15	.15	.12	.13				
26. <i>P. roberti</i>	.12	.12	.13	.12	.12	.12	.13	.13	.11	.11	.11	.11	.12	.11	.11	.11	.13	.129	.13	.13	.13	.11	.11	.14	.13		

ACKNOWLEDGMENTS

We are grateful to P.S.D'Andrea, A.Junqueira, P.Albajar, and P.Borodin (IOC-FIOCRUZ), for helping in field expeditions; S.M.Franco (Museu

Nacional - Rio de Janeiro), for helping with museum collection; H.N.Seuánez (CPQ-INCA), for reviewing the manuscript and to J.R.Coura (IOC-FIOCRUZ), for financial support received. To Dr. J.L.Patton (MVZ-Berkeley) and an anonymous referee, for valuable

suggestions in a previous version of this manuscript. Brazilian Institute of Environment and Natural Resources (IBAMA) granted us licenses for collecting samples. Work supported by FIOCRUZ (Dept of Tropical Medicine), Fundação Rio-Zoo, Rio de Janeiro, CAPES, and CNPq (Brazil).

LITERATURE CITED

- ANISKIN, U.M., 1994. Evolutionary cytogenetics of chinchillas of Peruvian Amazonia (Rodentia, Echimyidae). In: HAYKA, M. (Ed.) **Mammals of Peruvian Amazonia**. Hayka: Moscou. p.62-82.
- BANDELT, H.J.; FOSTER, P. & RÖHL, A., 1999. Median-joining networks for inferring intraspecific phylogenies. **Molecular Biology and Evolution**, Oxford, **16**(1):37-48.
- BARROS, R.M.S., 1978. **Variabilidade cromossômica em Proechimys e Oryzomys (Rodentia) do Amazonas**. São Paulo. PhD Thesis. Universidade de São Paulo.
- BUENO, M.L. & GOMEZ-LAVERDE, M., 1993. Variación heterocromática en *Proechimys semispinosus* (Rodentia: Echimyidae) de la región pacífica Colombiana. **Caldasia**, Bogotá, **17**:333-340.
- DA SILVA, M.N.F., 1998. Four new species of spiny rats of the genus *Proechimys* (Rodentia: Echimyidae) from the western Amazon of Brazil. **Proceedings of the Biological Society of Washington**, Washington, **11**:436-471.
- GARDNER, A.L. & EMMONS, L., 1984. Species group in *Proechimys* (Rodentia: Echimyidae) as indicated by karyology and bullar morphology. **Journal of Mammalogy**, Provo, **65**:10-25.
- GOMEZ-LAVERDE, M.; BUENO, M.L. & CADENA, A., 1990. C. Poblaciones de ratas (*Proechimys semispinosus*) (Rodentia: Echimyidae). In: AGUIRRE, J. & RANGEL, O. (Eds.) **Biota y Ecosistema de Gorgona y Gorgonilla**. Bogotá: Fondo Fen Colombia, Editorial Presencia. p.244-251.
- IKEUCHI, T., 1984. Inhibitory effect of ethidium bromide on mitotic chromosome condensation and its application to high resolution chromosome banding. **Cytogenetics and Cell Genetics**, Basel, **38**:35-61.
- KUMAR, S.; TAMURA, K.; JAKOBSEN, I.B. & NEI, M., 1993. MEGA2: molecular evolutionary genetics analysis software. **Bioinformatics**, Oxford, **17**(12):1244-1245.
- LEAL-MESQUITA, E.R.R.B.P., 1991. **Estudos citogenéticos em dez espécies de roedores brasileiros da família Echimyidae**, São Paulo. Ms. Dissertation. Universidade de São Paulo.
- MAIA, V. & LANGGUTH A., 1993. Constitutive heterochromatin polymorphism and NORs in *Proechimys cuvieri* Petter, 1978. **Brazilian Journal of Genetics**, Rio Preto, **16**:145-154.
- MATOCQ, M.D.; PATTON, J.L. & DA SILVA, M.N., 2000. Population genetic structure of two ecologically distinct Amazonian spiny rats: separating history and current ecology. **Evolution: International Journal of Organic Evolution**, Lawrence, **54**(4):1423-1432.
- MOOJEN, J., 1948. Speciation in the Brazilian spiny rats (genus *Proechimys*, family Echimyidae). **University of Kansas Publications**, Lawrence, **1**:301-406.
- PATTON, J.L., 1987. Species group of spiny rats, genus *Proechimys* (Rodentia, Echimyidae). **Fieldiana Zoology**, ns, Chicago, **39**:305-345.
- PATTON, J.L. & GARDNER, A.L., 1972. Notes on systematics of *Proechimys* (Rodentia: Echimyidae), with emphasis on Peruvian forms. **Occasional Papers Museum Zoology, Louisiana State University**, Baton Rouge, **44**:1-30.
- PATTON, J.L.; DA SILVA, M.N.F & MALCON, J.R., 2000. Mammals of the Rio Juruá and the evolutionary and ecological diversification of Amazonia. **Bulletin American Museum of Natural History**, New York, **244**:1-306.
- POSADA, D. & CRANDALL, K.A., 2001. Intraspecific gene genealogies: trees grafting into networks. **Trends in Ecology and Evolution**, Oxford, **16**(1):37-44.
- REIG, O.A. & USECHE, M., 1976. Diversidad cariotípica y sistemática en poblaciones venezolanas de *Proechimys* (Rodentia, Echimyidae), con datos adicionales sobre poblaciones de Perú y Colombia. **Acta Científica Venezolana**, Caracas, **27**:132-140.
- REIG, O.; TRAINER, M. & BARROS, M.A., 1979. Sur l'identification chromosomique de *Proechimys guyannensis* (E. Geoffroy, 1803) et de *Proechimys cuvieri* Petter, 1978 (Rodentia, Echimyidae). **Mammalia**, Paris, **43**:501-505.
- SAMBROOK, J.; FRITSCH, E.F. & MANIATIS, T., 1989. **Molecular Cloning: A Laboratory Manual**. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
- SEABRIGHT, M., 1971. A rapid banding technique for human chromosomes. **Lancet**, London and New York, **2**:971-972.
- SUMNER, A.T., 1972. A simple technique for demonstrating centromeric heterochromatin. **Experimental Cell Research**, Bath, **75**:304-306.
- SMITH, M.F. & PATTON, J.L., 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. **Biological Journal of Linnean Society**, London, **50**:149-177.
- SWOFFORD, D.L., 1999. "PAUP*: Phylogenetic Analysis Using Parsimony (and other methods), version 4.1", Washington: Smithsonian Institution Press.
- THOMAS, O., 1928. The Goldman-Thomas expedition to Peru. VII. The mammals of the Rio Ucayali. **Annals and Magazine of Natural History**, London, series 10, **2**:249-265.
- WEKSLER, M.; BONVICINO, C.R.; OTAZÚ, I.B. & SILVA JR., J.S., 2001. The status of *Proechimys roberti* and *Proechimys oris* (Rodentia: Echimyidae) from Eastern Amazonia and Central Brazil. **Journal of Mammalogy**, Provo, **82**(1):109-122.